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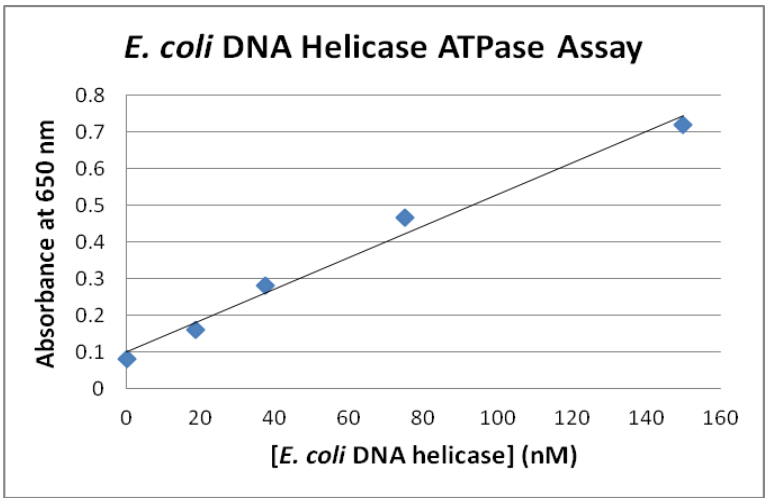
INSTRUCTIONS

ProFoldin
E.coli DNA Helicase ATPase Assay Kits

***E. coli* DNA Helicase ATPase assay Kit Plus-100** **Catalog No. DNAB100KE**
***E. coli* DNA Helicase ATPase assay Kit Plus-500** **Catalog No. DNAB500KE**

Introduction

DNA helicase (DnaB) hydrolyzes ATP as the source of molecular energy to carry out DNA unwinding required by the DNA replication process. Inhibition of the ATPase activity of DNA helicase blocks its DNA unwinding function. The DNA helicase ATPase assay can be used for high-throughput screen of DNA helicase inhibitors in drug discovery. The **DNA Helicase ATPase Assay Kit** is based on detection of the phosphate produced by the ATP hydrolysis reaction in the presence of DNA. The assay is in a 384-well plate format and the phosphate is detected using light absorbance at 650 nm.



The ***E. coli* DNA Helicase ATPase assay Kit Plus-100** (Catalog No. DNAB100KE) includes 500 µl of 10 x assay buffer, 35 µl of 100 x DNA, 30 µl of 100 x *E. coli* DNA helicase, 35 µl of 100 x ATP and 5 ml of dye for 100 assays in a 384-well assay format.

The ***E. coli* DNA Helicase ATPase assay Kit Plus-500** (Catalog No. DNAB500KE) includes 2 ml of 10 x assay buffer, 170 µl of 100 x DNA, 150 µl of 100 x *E. coli* DNA helicase, 170 µl of 100 x ATP and 25 ml of dye for 500 assays in a 384-well assay format.

Publication

Nakano T., et al, Translocation and Stability of Replicative DNA Helicases upon Encountering DNA-Protein Cross-links, J. Biol. Chem. 288: 4649-4658 (2013).

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Assay Protocol

1. Reagent preparation:

For each 10 assay reactions,

(1) Prepare 297 µl of premix composed of 261 µl of H_2O, 33 µl of 10 x Buffer and 3.3 µl of 100 x *E. coli* DNA helicase.

(2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x ATP and 3.3 µl of 100 x DNA and 26.4 µl of water.

2. Reaction:

Mix 27 µl of the premix with 3 µl of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations for the ATPase assays of the helicases are 20 mM HEPES, pH 7.5, 20 mM potassium glutamate, 1 mM DTT, 0.005% Triton X-100, 10 mM $MgCl_2$, 250 nM DNA, 0.25 mM ATP and 200 nM DNA helicase. A negative control reaction can be the reaction mixture without addition of ATP or enzyme.

3. Detection:

Add 45 µl of the Dye MPA3000 into the 30 µl of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

Assay optimization for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at http://www.profoldin.com/services.html.

Related products

H. influenzae DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KH
S. pneumoniae DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KN
P. aeruginosa DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KP
S. aureus DNA Helicase ATPase assay Kit Plus-100	Catalog No. DNAB100KS
E. coli DNA Primase Assay Kit Plus	Catalog No. EGA100KE
H. influenzae DNA Primase Assay Kit Plus	Catalog No. HGA100KE
S. pneumonia DNA Primase Assay Kit Plus	Catalog No. PGA100KE
S. aureus DNA Primase Assay Kit Plus	Catalog No. AGA100KE

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.